

DYNORPHIN REDUCES VOLTAGE-DEPENDENT CALCIUM CONDUCTANCE OF MOUSE
DORSAL ROOT GANGLION NEURONS

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ABSTRACT

Dynorphin A (DYN)(1 μ M) decreased somatic calcium-dependent action potential (CAP) duration of a portion of dorsal root ganglion (DRG) neurons in a naloxone reversible manner. Responses to DYN differed from responses to Leu-enkephalin in that only DYN decreases of somatic CAP duration were associated with decreased action potential after hyperpolarization and persisted after intracellular injection of the potassium channel blocker cesium. While Leu-enkephalin at 10 μ M did not affect somatic CAP duration of DRG neurons impaled with cesium-filled micropipettes, dynorphin A (1-8), dynorphin B, and β -neoendorphin were effective at 1 μ M. During single electrode voltage clamp, DYN decreased inward current in a portion of DRG neurons under conditions that predominately isolated calcium current. Leak current was unaffected by dynorphin A. Therefore, we suggest that DYN decreases voltage-dependent calcium conductance. The action on calcium conductance appears specific for opioids with affinity for kappa-receptors.

INTRODUCTION

In dorsal horn of the spinal cord, approximately 50% of opioid binding is thought to occur on primary afferents (1,2). Consistent with this observation, opioids have been reported to depress neurotransmitter release from primary afferent terminals (3,4,5). In addition to being localized on primary afferents, opioid receptors are present on the somata of dorsal root ganglion(DRG) neurons in vivo (6) and also grown in primary dissociated cell culture (5,7). Binding of opioids to receptors on DRG neuron somata has been shown to result in a decrease of calcium-dependent action potential (CAP) duration (5,7). Interestingly, a similar effect of opioids on calcium entry at DRG neuron terminals would result in decreased neurotransmitter release and, thus, opioid receptors on DRG neuron somata and terminals are likely to be functionally similar. In this study we compare the actions of dynorphin A (DYN) and Leu-enkephalin (L-ENK) on DRG neuron somatic CAPs and calcium-dependent currents.

METHODS

Preparation of mouse spinal cord and DRG co-cultures and electrophysio-

logical techniques were as previously described (7). Opioid effects on somatic CAP duration were assessed on neurons bathed in a Tris-HCl buffered (pH 7.2-7.4) balanced saline (320 mOsm) containing (in mM): NaCl, 137; KCl, 5.3; MgCl₂, 0.8; CaCl₂, 5.0; Tris-base, 13.0; glucose, 5.6, and tetraethylammonium chloride (TEA), 5.0. DRG neurons were impaled with either 4M potassium acetate (KAc) or 4 M cesium acetate (CsAc)-filled micropipettes (20-40 M Ω). For voltage clamp experiments, the recording medium was the same except that potassium was replaced with cesium, and neurons were impaled with micropipettes (15 - 25 M Ω) containing 3M CsCl. Intracellular cesium blocks potassium conductance and thus predominately isolated the calcium current. DRG neurons were single-electrode voltage-clamped using an Axoclamp preamplifier (Axon Instruments, CA, USA) that switched between voltage recording (70% of each duty cycle) and current passing (30% of each duty cycle) at 6 KHz. Opioid Peptides (Peninsula) were applied to single neurons by pressure ejection from micropipettes with tip diameters of 2-5 μ m. Naloxone and cadmium were applied by diffusion from 15-25 μ m micropipettes.

RESULTS

We compared the actions of 1 μ M DYN and 10 μ M L-ENK on single DRG neurons recorded initially with KAc-filled micropipettes and subsequently with CsAc-filled micropipettes. During KAc recording, some DRG neurons responded only to DYN (n=16), others responded to DYN and L-ENK (n=5), and 25 neurons responded to neither peptide. DYN decreases of CAP duration were associated with decreased after hyperpolarization while while L-ENK augmented action potential after hyperpolarization. DYN responses also differed from L-ENK responses in that only DYN responses persisted following intracellular cesium injection. While L-ENK was ineffective in decreasing CAP duration of DRG neurons impaled with CsAc-filled micropipettes at 10 μ M, dynorphin A(1-8), dynorphin B, and β -neoendorphin were effective at 1 μ M.

The single electrode voltage clamp technique was used to directly assess

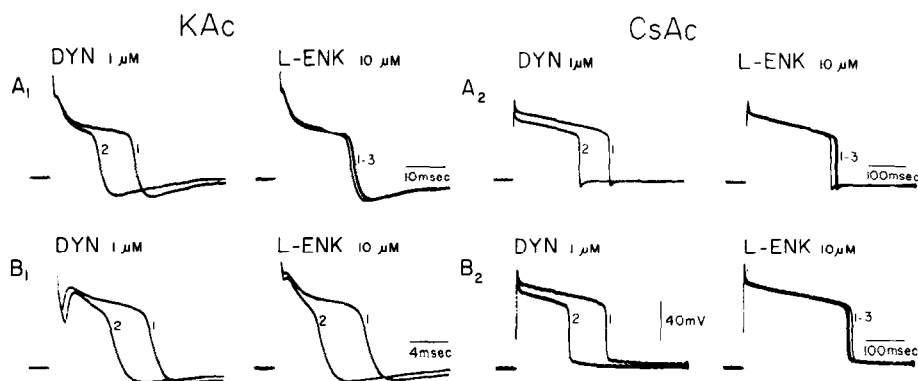


Fig. 1 DRG neuron responses to DYN and L-ENK were heterogeneous. In this and the following figure, action potentials were evoked at a frequency of 4 per min by 100 μ sec depolarizing pulses. Action potentials evoked prior to (1) and subsequent to (2) opioid peptide application were superimposed. A and B: a DRG neuron that responded well to DYN but not to L-ENK (A₁) and a DRG neuron that responded to both opioids (B₁) during recording with KAc-filled micropipettes. Subsequent reimpementation of the neurons with CsAc-filled micropipettes did not attenuate responses to DYN in either case (A₂, B₂). Although DYN responses persisted following intracellular injection of cesium, L-ENK responses were blocked.

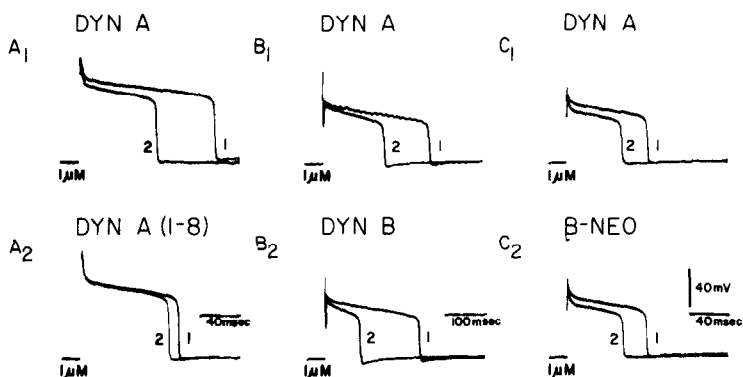


Fig. 2. DYN A, dynorphin A(1-8), dynorphin B, and β -neoendorphin decreased CAP duration of DRG neurons impaled with CsAc-filled micropipettes. A, B, and C are recordings from three neurons showing the potency of opioids at $1 \mu\text{M}$ relative to DYN A.

DYN action on calcium currents when potassium conductances were minimized by injection of intracellular and addition of extracellular cesium. Step depolarizations of neurons held at -60 mV to potentials between -40 to $+25 \text{ mV}$ produced net inward currents 1 - 10 nA in amplitude that only partly decayed over 75 msec . The magnitude of the inward current was dependent upon extracellular calcium concentration and was abolished by the calcium channel blocker cadmium. DYN at $1 \mu\text{M}$ reversibly decreased the magnitude of the depolarization evoked inward currents in 13 of 38 neurons (Fig. 3A). In contrast, L-ENK at $10 \mu\text{M}$ did not affect inward current amplitude in 11 of 11 neurons including 5 neurons that did respond to DYN. The DYN reduction of inward current was antagonized by naloxone ($n=5$) (Fig. 3B). When the inward calcium current was blocked by cadmium, the remaining leak current was unaffected by DYN (Fig. 3).

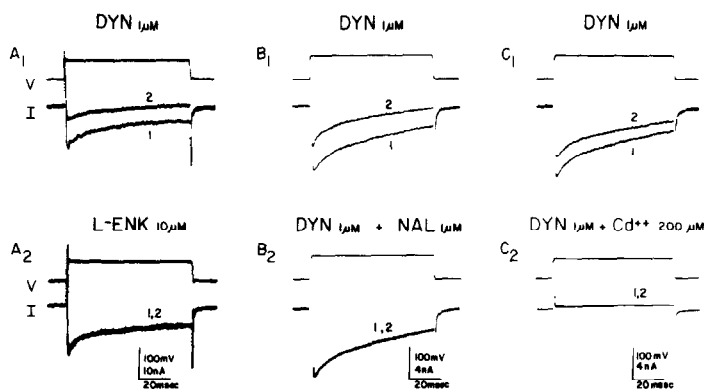


Fig. 3. DYN decreased calcium-dependent inward currents. During single electrode voltage clamp, step depolarizations (V) from a holding potential of -60 mV evoked inward currents (I). A: Superimposed currents obtained prior to (1) and subsequent to opioid application are shown. DYN (A_1) but not L-ENK decreased the amplitude of calcium-dependent inward currents. Naloxone antagonized the reduction of inward current by DYN (B). C: DYN decreased inward current when potassium conductances were largely blocked by cesium, but did not affect leak currents when the inward calcium current was blocked by $200 \mu\text{M}$ cadmium.

DISCUSSION

We observed that DYN decreased somatic CAP duration in a subpopulation of DRG neurons. DYN action was antagonized by naloxone indicating an interaction with opioid receptors. We suggest, based on three observations, that binding of DYN to this receptor results in a decrease of voltage-dependent calcium conductance. Firstly, DYN decreased CAP after-hyperpolarization indicating that potassium conductance was unlikely to be enhanced. Secondly, DYN decreases of CAP persisted when substantial potassium conductance was blocked by intracellular cesium. Thirdly, DYN reduced depolarization-evoked calcium currents but did not alter membrane conductance following blockade of calcium channels by cadmium. The reduction of inward current was observed in neurons bathed in recording medium substituting cesium for potassium and impaled with CsCl-filled micropipettes. Thus, the DYN effect was observed when the calcium current was predominately isolated from potassium currents. The reduction of calcium conductance appears specific for DYN and other opioids, such as DYN A(1-8), dynorphin B and β -neoendorphin, with affinity for kappa-opioid receptors. In contrast, L-ENK, which lacks affinity at kappa-receptors, appears to act by enhancing potassium conductance (8).

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